

## REVIEW

10.1111/j.1469-0691.2008.02081.x

## Can pharmacokinetic–pharmacodynamic parameters provide dosing regimens that are less vulnerable to resistance?

*P. Courvalin**Unité des Agents Antibactériens, Institut Pasteur, Paris, France*

### ABSTRACT

Dissemination of antibiotic resistance in bacteria is associated with prescription of the corresponding drugs. Various pharmacokinetic–pharmacodynamic parameters have been developed with the intention of reducing the spread of resistance. In this review, it is considered whether dosing regimens based on these parameters can delay this spread. The evolution of bacterial resistance to antibiotics involves two successive but distinct and independent mechanisms. The first occurs by mutation in the genome, including the host chromosome and mobile accessory genetic elements such as plasmids or transposons, or, following acquisition of a resistance determinant from another bacterium, by horizontal gene transfer. These two genetic events happen by chance, which means that they do not rely on the presence of an antibiotic in the environment; that is, they are not induced, but simply revealed and propagated by the drugs. The second step is dissemination of resistance which can be due to the spread of bacteria (clonal epidemics), of replicons (plasmid epidemics) or of resistance determinants (gene epidemics). Resistance dissemination by each one of these three levels which superimpose in nature, is not only infectious but also exponential, since all three are associated with DNA replication (duplication) of the host chromosome, of a plasmid, or of a transposon. As opposed to emergence, dissemination is clearly associated with the selective pressure exerted by antibiotic prescription [1,2]. The consequence of this dual evolutionary pathway is that proper use of antibiotics will, at best, delay the spread of resistance. In this review, the pharmacokinetic–pharmacodynamic (PK–PD) parameters that are intended to lower resistance dissemination are considered exclusively.

**Keywords** antibiotic, bacterium, pharmacodynamic, pharmacokinetic, resistance, review

**Accepted:** 5 August 2008

*Clin Microbiol Infect* 2008; **14**: 989–994

### COMMON-SENSE ACRONYMS

Two notions have been introduced into the field of pharmacokinetics–pharmacodynamics (PK–PD), with the intent of fighting resistance (for a recent review, see Ref. [3]): the mutation prevention concentration (MPC) and the mutation selection window (MSW) (Fig. 1). The MPC, as its name indicates, is the concentration of antibiotic that prevents selection (not of emergence, as already discussed) of resistant mutants. What this acronym actually means is that, to kill a bacterium,

what is needed is more antibiotic than the lowest concentration that would inhibit this bacterium (a value known as the minimal inhibitory concentration, MIC)—a rather obvious statement! Thus, and as its name does not indicate, the MPC corresponds to the MIC of the mutant. In the case of stepwise acquisition of resistance by successive mutations, one then ends up with MPC1, MPC2, etc., which correspond, respectively, to the MICs of first-step, second-step, etc. mutants. The MSW is the range of antibiotic concentrations that optimally favours growth of the resistant mutants. These concentrations range from sub-MIC (close to MIC) values for the susceptible parental strain to the MIC for the mutant derivatives, the so-called MPC. This is a notion that has been known for a very long period of time and has been exploited by

Corresponding author and reprint requests: P. Courvalin, Unité des Agents Antibactériens, Institut Pasteur, 25–28 rue du Docteur Roux, 75724 Paris Cedex 15, France  
E-mail: [pcourval@pasteur.fr](mailto:pcourval@pasteur.fr)

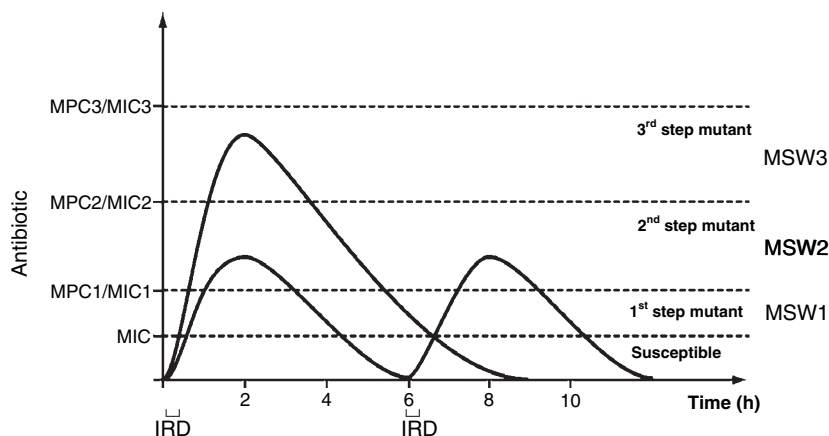


Fig. 1. Pharmacokinetic curves after one or two administration(s) of an antibiotic. MIC, minimal inhibitory concentration; MPC, mutation prevention concentration; MSW, mutation selection window; IRD, induction revolving door.

geneticists in 56-year-old techniques [4]. As with MPCs, there are MSW1, MSW2, etc.

According to this 'concept', in a treated infection, the bacteria spend their time watching at the window, so to speak, the susceptible bacteria at MSW1, the first-step mutants at MSW2, the second-step mutants at MSW3, and so on.

### THE SUBTLE CHARMS OF ACRONYMS

The notions of MPC and MSW were rediscovered *à propos* the very simple and narrow system of fluoroquinolone resistance by mutational alteration of the drug targets, the type II topoisomerases, DNA gyrase and topoisomerase IV [5]. Even in this over-simplistic system, it has recently been shown that selection of resistant commensals during therapy is not preventable by optimizing the dosing regimen (47th Interscience Conference on Antimicrobial Agents and Chemotherapy 2007, abstract 585). These two acronyms cannot even be applied to Gram-negative bacteria, because of plasmid-mediated resistance to this class of molecules through target protection [6,7], inactivation [8] or efflux [9].

In this simplistic resistance system, there is an infinite number of MPCs! This is due to:

- 1 The multiplicity of quinolones, which differ in their intrinsic activity. A resistance mechanism has no intrinsic value; it simply increases the concentration of antibiotic at which the bacterium is inhibited. Thus, the more active the fluoroquinolone, the lower the level of resistance of a given type of mutant, and thus the lower the drug concentrations that correspond to the MPC [10].

- 2 The multiplicity of bacterial species that differ in their susceptibility to a given antibiotic. For example, the MIC of ciprofloxacin for *Pseudomonas aeruginosa* (0.5 mg/L) is clearly very different from that for *Neisseria meningitidis* (0.0015 mg/L). This implies that MPCs are not only genus-specific but even species-specific. They are, in fact, strain-specific!
- 3 The fact that the activity of antibiotics is determined *in vitro* on planctonic cells, whereas they often generate biofilms *in vivo*. This change in lifestyle can greatly affect bacterial susceptibility to antibiotics and is not taken into account in the PK-PD approach.
- 4 The fact that, as is increasingly being shown, a large number of bacteria can be, at least transiently, located intracellularly, where the antibiotic concentrations are clearly distinct from those in the extracellular compartments, an observation that should also be taken into consideration.
- 5 The commensal flora adding to this already complicated situation. When one treats a patient infected with a pathogenic bacterium with an antimicrobial agent, although therapy is intended to eradicate the pathogen, the antibiotic acts on the entire flora of the patient; in particular, in the case of oral administration, high concentrations of drugs are in contact with the gigantic population of commensals in the digestive tract. It has been recently proposed that resident gut bacteria could act as a reservoir of resistance for more pathogenic species [11]. It would thus be advisable to determine MPC-MSW values for digestive commensals, including anaerobes, an impossible task.

- 6 The MPC-MSW quantification relying on antibiotic concentrations that are amenable to dosages, that is, in practice, mainly blood levels. This is not particularly informative or useful, insofar as the relevant values are those at the site of infection, where action takes place; these are most often inaccessible.

So, not only is there an infinite number of MPC-MSW values, but PK-PD has also generated a few additional 'predictive indices', such as  $C_{\max}/\text{MIC}$ ,  $C_{\max}/\text{MPC}$ , time above MPC, and time at the window. [3]. The mere multiplicity of these indices indicates that none of them is of general value.

### USELESS ACRONYMS

The uselessness of PK-PD to contribute to the diminution of resistance is stressed by the following facts.

- 1 Colonization of patients by strains that are already resistant is by far more common than mutant selection under therapy [10]. An example of this is fluoroquinolone resistance in methicillin-resistant *Staphylococcus aureus* (MRSA), and it also accounts for the impact of the pneumococcal vaccine on antibiotic resistance in this genus, not only for the vaccinees, but also for their close contacts [12].
- 2 Under natural conditions, regulatory mutations are more frequent, and thus more clinically relevant, than target mutations [13]. In addition, the former type of genetic event can confer nearly a continuum of resistance levels, depending upon the type or locus of the mutation [13]. This, in turn, generates a continuum of MPC-MSWs.
- 3 Resistance is much more likely to occur following horizontal gene transfer than by mutation. Becoming resistant to an antibiotic corresponds to acquisition of a function by the bacterium. The price to pay for this new function is known as the biological cost of resistance. As bacteria are subjected to the principle of parsimony, they have to minimize the cost of resistance to maintain a degree of fitness close to that of the parental susceptible strain, in order to remain competitive with the susceptible bacterial population in the absence of antibiotic. An efficient and elegant way to maintain fitness is to harbour an inducible resistance mechanism;

in other words, resistance that will be phenotypically expressed exclusively in the presence of the antibiotic, i.e. when needed [13].

- 4 An additional observation is resistance by efflux of the drugs. Sequence determination of the genomes of representatives of multiple bacterial genera has revealed that they all encode efflux pumps that expel, among other toxic molecules, antibiotics [14]. Expression of the structural genes for these pumps, which can be considered as bacterial kidneys, is regulated in a tight fashion, positively or negatively [13]. Clinical isolates that became resistant by gene overexpression following a mutation in the regulatory modules for a pump have been reported [15]. In general, efflux pumps, and in particular members of the RND superfamily [16], have very broad substrate ranges, including various drug classes as well as biocides [17]. Thus, a single regulatory mutation corresponds to a very large number of MPCs and MSWs, rendering impossible the prevention of its occurrence.

Antibiotic resistance is only transiently useful to the bacteria. This makes horizontal acquisition of inducibly regulated genetic information much more effective in facing the challenge of an antibiotic in the environment than the occurrence of mutations that are, at best, slowly reversible. Consideration of populations, rather than individual bacteria, confirms that acquisition of a mobile accessory genetic element fulfils the requirement for transient (multi)drug resistance. Most unfortunately, neither MPCs nor MSWs can influence the rate of transfer by the mechanisms discussed below, which are increased by the presence of antibiotics in the bacterial ecosystems.

### FACILITATION OF CONJUGATIVE RESISTANCE TRANSFER

DNA translocation across bacterial membranes occurs at the early stages of conjugation. The peptidoglycan of Gram-positive bacteria is 50–100 layers thick. Thus, the cell wall of these bacteria might constitute an important barrier for the acquisition of exogenous DNA. Consistently, it has been shown that sub-MIC concentrations of penicillins (oxacillin or penicillin G) in the mating medium results in a 50-fold

increase of the conjugal transfer of plasmid DNA from *Escherichia coli* to *S. aureus* and *Listeria monocytogenes* [18]. Similarly, it has been reported that low oxacillin concentrations increase the *in vitro* frequency of transfer of the enterococcal conjugative transposon Tn916 from *Enterococcus faecalis* to *Bacillus anthracis* [19]. Penicillins decrease the percentage of peptidoglycan cross-linkage by inhibition of the transpeptidases, which might increase cell permeability and facilitate the formation of mating aggregates. In addition, cell wall damage caused by incorporation of cell wall-active agents into the culture medium similarly improves the efficiency of electro-transformation of Gram-positive bacteria [17,20–22].

### INDUCTION OF CONJUGATIVE RESISTANCE TRANSFER

Some transposons have evolved such that the antibiotic to which they confer resistance can specifically stimulate their intercellular mobility. Conjugative transposons (or integrative conjugative elements) of the Tn916/Tn1545 family are widespread in Gram-positive cocci and contain the *tet*(M) tetracycline resistance determinant, either alone (e.g. Tn916) or associated with other resistance genes (e.g. Tn1545) [23–25]. Exposure of bacteria harbouring Tn1545 to low concentrations (0.2–1 mg/L) of tetracycline results in a 10-fold to 100-fold increase in its transfer frequency both *in vitro* and in the digestive tract of gnotobiotic mice [26]. This observation has been confirmed for other elements [27], including the Tn916 prototype [28]. Similarly, low levels of tetracycline stimulate the transfer of the *Bacteroides* conjugative Tc<sup>r</sup> Em<sup>r</sup> 10 000-fold [29]. By a totally different molecular mechanism, the SOS response to DNA damage alleviates repression of the transfer functions of the *c.* 100-kb element SXT. This integrative conjugative element, which is common in various bacterial species and, in particular, in *Vibrio cholerae*, confers resistance to chloramphenicol, streptomycin, sulphonamides and trimethoprim. The SOS response can be triggered by a variety of environmental factors, including antibiotics, and it has been shown that SOS response induction by low concentrations of fluoroquinolones and mitomycin C markedly enhances the transfer of SXT and related genetic elements [30].

### INDUCTION OF TRANSFORMATION IN *STREPTOCOCCUS PNEUMONIAE*

Natural transformation is the ability of bacteria that belong to a certain species (*Streptococcus pneumoniae*, *Acinetobacter* spp., *Neisseria* spp.) and are in a competent state to pick up naked DNA from the environment [31]. It has been shown recently that low concentrations of certain aminoglycosides or fluoroquinolones can very efficiently induce competence in *Streptococcus pneumoniae* in the absence of bacterial killing [32]. It therefore appears that the stress induced by antibiotics increases the rate of genetic exchange, including that of genes mediating antibiotic resistance. Again, in this transfer system, no MPC or MSW can impede enhancement of the spread of resistance in pneumococci associated with antibiotic therapy.

### THE INDUCTION REVOLVING DOOR

As already mentioned, the majority of resistance mechanisms are inducibly expressed. This is the case, for example, for VanA-type vancomycin resistance in enterococci [13]. Induction of resistance is due to the presence, upstream from the resistance genes, of two genes that encode a two-component regulatory system. This system, which is very common in bacteria, is responsible for activation of transcription of the resistance genes, which occurs at very low concentrations of vancomycin or teicoplanin. Acquisition by MRSA of the *vanA* operon from enterococci led to two types of VanA-type MRSA [33,34]. One half of the clinical isolates is resistant to high levels of both glycopeptides, whereas the remaining half is resistant to low levels of vancomycin and remains susceptible to teicoplanin. The latter phenotype is due to two interlinked phenomena: a high rate of resistance loss due to plasmid instability, and a very long delay in induction of resistance, resulting in a prolonged lag phase (Fig. 2). This observation is important because: (i) on an individual (patient) basis, resistance being inducible, glycopeptide therapy will lead to clinical failure; and (ii) on a community basis, the second type of strain remains undetected (in particular, by automated susceptibility systems), and this delays the implementation of hygiene measures to prevent further spread of these dangerous microorganisms. Therefore, the very low concentration of

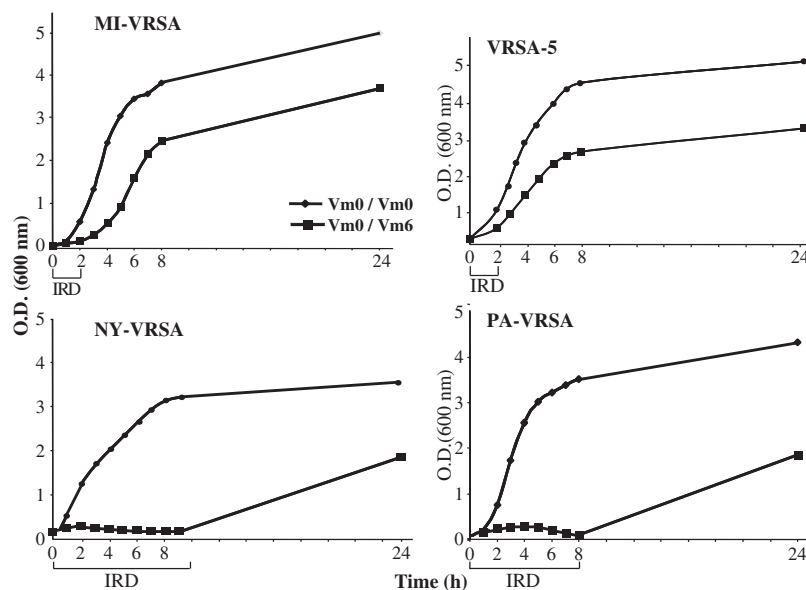


Fig. 2. Growth of VanA-type methicillin-resistant *Staphylococcus aureus* in the absence (Vm0) or in the presence of 6 mg/L vancomycin (Vm6) after overnight culture with or without vancomycin (6 mg/L). The lines indicate the ratio of the vancomycin concentration in the overnight culture/vancomycin concentration in the culture medium. IRD, induction revolving door.

antibiotics at the onset of therapy is crucial for induction of clinical resistance by various mechanisms [13]. Most curiously, these initial and repeated low levels of antibiotics are ignored in the PK-PD approach, which is intended to minimize resistance. Because, as we have seen, in PK-PD representation the windows are already occupied, we are left with (and at) the door of the antibiotic kinetic. I thus propose the notion of induction door (ID) for this portion of the curve (Fig. 1). Although the acronym sounds good (and in fact may well represent the only sensible idea in this entire story), MIC, MPC and MSW strongly suggest that, to be successful, an acronym should comprise three letters. Considering that, during induction, the antibiotic is neither consumed nor destroyed but is recycled, I propose the concept of induction revolving door (IRD).

## CONCLUSION

### Votre fille est muette

As we have seen repeatedly in this brief review, the PK-PD acronyms dealing with antibiotic resistance are remarkable truisms. They are reminiscent of the famous prototype in the medical field 'votre fille est muette' [35]. In addition, they introduce confusion by rediscovering old, well-established and well-accepted notions and by generating unnecessary acronyms (e.g. MPC for MIC).

If, as a medical bacteriologist, you are short of ideas or unable to contribute a new concept to the field, I suggest that you coin an acronym. As I have tried to point out, the latter does not need to convey any new notion to be popularized and you may well end up being remembered.

## ACKNOWLEDGEMENTS

I thank the ECCMID organizing committee for having given me the opportunity to present these ideas during the ECCMID, and PE Reynolds for reading the manuscript.

## TRANSPARENCY DECLARATION

The author declares no sources of funding and no conflicts of interest.

## REFERENCES

1. Dowell SF, Schwartz B. Resistant pneumococci: protecting patients through judicious use of antibiotics. *Am Fam Physician* 1997; **55**: 1647-1654.
2. Chen DK, McGeer A, de Azavedo JC, Low DE. Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. Canadian Bacterial Surveillance Network. *N Engl J Med* 1999; **341**: 233-239.
3. Drlica K, Zhao X. Mutant selection window hypothesis updated. *Clin Infect Dis* 2007; **44**: 681-688.
4. Szybalski W, Bryson J. Genetic studies on microbial cross resistance to toxic agents. I. Cross resistance of *Escherichia coli* to fifteen antibiotics. *J Bacteriol* 1952; **64**: 489-499.
5. Hawkey PM. Mechanisms of quinolone action and microbial response. *J Antimicrob Chemother* 2003; **51**: 29-35.



6. Martínez-Martínez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. *Lancet* 1998; **351**: 797–799.
7. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect Dis* 2006; **6**: 629–640.
8. Robicsek A, Strahilevitz J, Jacoby GA *et al.* Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat Med* 2006; **12**: 83–88.
9. Périchon B, Courvalin P, Galimand M. Transferable resistance to aminoglycosides by methylation of G1405 in 16S rRNA and to hydrophilic fluoroquinolones by QepA-mediated efflux in *Escherichia coli*. *Antimicrob Agents Chemother* 2007; **59**: 459–464.
10. Courvalin P, Trieu-Cuot P. Minimizing potential resistance: the molecular view. *Clin Infect Dis* 2001; **33**: S138–S146.
11. Launay A, Ballard SA, Johnson PDR, Grayson ML, Lambert T. Transfer of vancomycin resistance transposon Tn1549 from *Clostridium symbiosum* to *Enterococcus* spp. in the gut of gnotobiotic mice. *Antimicrob Agents Chemother* 2006; **50**: 1054–1062.
12. Kyaw MH, Lynfield R, Schaffner W *et al.* Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. *N Engl J Med* 2006; **354**: 1455–1463.
13. Depardieu F, Podglajen I, Leclercq R, Collatz E, Courvalin P. Modes and modulations of antibiotic resistance gene expression. *Clin Microbiol Rev* 2007; **20**: 79–114.
14. Saier MH Jr. Computer-aided analyses of transport protein sequences: gleanings concerning function, structure, biogenesis, and evolution. *Microbiol Rev* 1994; **58**: 71–93.
15. Marchand I, Damier-Piolle L, Courvalin P, Lambert T. Expression of the RND-type efflux pump AdeABC in *Acinetobacter baumannii* is regulated by the AdeRS two-component system. *Antimicrob Agents Chemother* 2004; **48**: 3298–3304.
16. Nikaido H. Multidrug efflux pumps of gram-negative bacteria. *J Bacteriol* 1996; **178**: 5853–5859.
17. McMurtry LM, Oethinger M, Levy S. Overexpression of *marA*, *sosS*, or *acrAB* produces resistance to triclosan in laboratory and clinical strains of *Escherichia coli*. *FEMS Microbiol Lett* 1998; **166**: 305–309.
18. Trieu-Cuot P, Derlot E, Courvalin P. Enhanced conjugative transfer of plasmid DNA from *Escherichia coli* to *Staphylococcus aureus* and *Listeria monocytogenes*. *FEMS Microbiol Lett* 1993; **109**: 19–23.
19. Ivins BE, Welkos SL, Knudson GB, Leblanc DJ. Transposon Tn916 mutagenesis in *Bacillus anthracis*. *Infect Immun* 1988; **56**: 176–181.
20. Cruz-Rodz AL, Gilmore MS. High efficiency introduction of plasmid DNA into glycine treated *Enterococcus faecalis* by electroporation. *Mol Gen Genet* 1990; **224**: 152–154.
21. Dunny GM, Lee LN, LeBlanc DJ. Improved electroporation and cloning vector system for gram-positive bacteria. *Appl Environ Microbiol* 1991; **57**: 1194–1201.
22. Park SF, Stewart GS. High-efficiency transformation of *Listeria monocytogenes* by electroporation of penicillin-treated cells. *Gene* 1990; **94**: 129–132.
23. Clewell DB, Flannagan SE, Jaworski DD. Unconstrained bacterial promiscuity: the Tn916–Tn1545 family of conjugative transposons. *Trends Microbiol* 1995; **3**: 229–236.
24. Salyers AA, Shoemaker NB, Stevens AM, Ly L. Conjugative transposons: an unusual and diverse set of integrated gene transfer elements. *Microbiol Rev* 1995; **59**: 579–590.
25. Scott JR, Churchward GG. Conjugative transposition. *Annu Rev Microbiol* 1995; **49**: 367–397.
26. Doucet-Populaire F, Trieu-Cuot P, Dosbaa I, Andremont A, Courvalin P. Inducible transfer of conjugative transposon Tn1545 from *Enterococcus faecalis* to *Listeria monocytogenes* in the digestive tracts of gnotobiotic mice. *Antimicrob Agents Chemother* 1991; **35**: 185–187.
27. Torres OR, Korman RZ, Zahler SA, Dunny GM. The conjugative transposon Tn925: enhancement of conjugal transfer by tetracycline in *Enterococcus faecalis* and mobilization of chromosomal genes in *Bacillus subtilis* and *E. faecalis*. *Mol Gen Genet* 1991; **225**: 395–400.
28. Showsh SA, Andrews RE Jr. Tetracycline enhances Tn916-mediated conjugal transfer. *Plasmid* 1992; **28**: 213–224.
29. Li LY, Shoemaker NB, Salyers AA. Location and characteristics of the transfer region of a *Bacteroides* conjugative transposon and regulation of transfer genes. *J Bacteriol* 1995; **177**: 4992–4999.
30. Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* 2003; **427**: 72–74.
31. Maynard Smith J, Dowson CG, Spratt BG. Localized sex in bacteria. *Nature* 1991; **349**: 29–31.
32. Prudhomme M, Attaiech L, Sanchez G, Martin B, Claverys JP. Antibiotic stress induces genetic transformability in the human pathogen *Streptococcus pneumoniae*. *Science* 2006; **313**: 89–92.
33. Périchon B, Courvalin P. Heterologous expression of the enterococcal *vanA* operon in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2004; **48**: 4281–4285.
34. Périchon B, Courvalin P. Synergism between  $\beta$ -lactams and glycopeptides against VanA-type methicillin-resistant *Staphylococcus aureus* and heterologous expression of the *vanA* operon. *Antimicrob Agents Chemother* 2006; **50**: 3622–3630.
35. Poquelin JB (Molière). *Le médecin malgré lui*. Acte 2, scène 4. Paris: Pocket, 1666; 62, 65.